## EXPERIMENTAL BIOLOGY

# EFFECT OF HELIUM-NEON LASER RADIATION ON MYOCARDIAL ENERGY METABOLISM

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The radiation of a helium—neon laser gives rise to general and local biological effects, has a vasodilator, antiinflammatory, analgesic action, stimulates the protective forces of the body, speeds up the rate of healing of ulcers and wounds, and stimulates enzyme activity and energy metabolism [1, 11]. The positive effect of irradiation with low-intensity lasers on the course of ischemic heart disease has now been demonstrated [6, 10, 13]. It has been shown that the effect of intravenous laser therapy in acute myocardial infarction resembles the effect of several drugs, and includes an antiarrhythmic and analgesic action, improvement of the rheologic parameters of the blood, and restoration of cardiomyocyte membrane function. These effects help to prevent complications in the acute period of myocardial infarction [6, 9, 10]. Interest in the problem of enhancing tissue resistance to hypoxic states through the effect of low-intensity laser radiation in the red region of the spectrum has recently increased. Reactivation of enzymes such as catalase and superoxide dismutase [2], activation of the microcirculation [8], and intensification of oxidation—reduction processes in the tissues are regarded as components of this phenomenon [3, 15]. It has been suggested that the appearance of vibratory excited states of molecules under the influence of low intensity laser irradiation leads to conformation of local areas of cell membranes and activation of individual enzyme systems [4].

Accordingly the aim of the present investigation was to study the effect of helium—neon laser irradiation on some histochemical parameters of myocardial energy metabolism. Enzymes involved in glycolysis, the Krebs' cycle, and terminal oxidation were investigated: glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and reduced nicotinamide-adenine dinucleotide dehydrogenase (NADH-DH).

#### **EXPERIMENTAL METHOD**

Experiments were carried out on noninbred male albino rats weighing 250 g. The animals were divided into three groups. In the rats of Group 1, a light guide was introduced through the jugular vein into the anterior vena cava, and the blood was irradiated for 30 min with a helium—neon laser, with wavelength of 632.8 nm. The LG-75 apparatus with output power of 2-4 mW on the light guide was used for this purpose. In the animals of Group 2, immediately after decapitation areas of the left ventricular myocardium were excised and irradiated directly in vitro with the helium—neon laser under the same conditions. Animals of Group 3 acted as the control. Each group consisted of nine animals. In the animals of Group 1, after decapitation the heart was quickly removed and the left ventricular myocardium was excized and frozen in liquid nitrogen. In Group 2 the myocardium was frozen immediately after irradiation. Histochemical tests for detection of G6PDH, LDH, SDH, and NADH-DH were conducted on frozen sections 7  $\mu$  thick [12]. The number of fibers with low, average, high, and very high activity was counted. The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney nonparametric test.

### **EXPERIMENTAL RESULTS**

The most active enzymes in the myocardium of the control animals were SDH and LDH, activity of NADH-DH was a little weaker, and that of G6PDH much weaker. During detection of these dehydrogenases, attention was drawn to the mosaic

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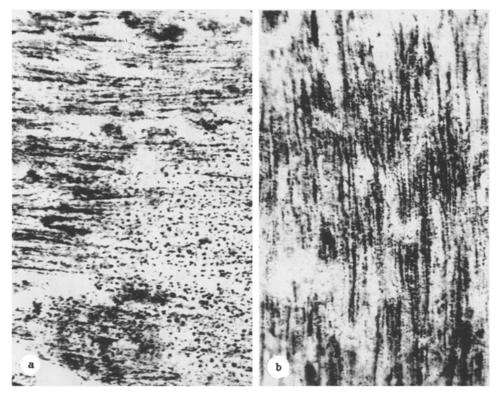


Fig. 1. Histochemical detection of G6PDH: a) control; b) after intravenous irradiation. 400×.

pattern of their distribution, as shown by different numbers of mono- and diformazan granules of different sizes both in neighboring cardiomyocytes and within the same cardiomyocyte. Mono- and diformazan granules of different sizes were arranged intracellularly, mainly in a linear manner along the myofibrils, emphasizing in some myocytes the cross-striation, whereas foci with a diffuse distribution of diformazan granules were found less frequently. Activity of these enzymes could not be detected in-nuclei of myocytes or in intercalated disks.

The test for G6PDH revealed low and, less frequently, average activity of the enzyme: a few medium-sized and large monoformazan granules, arranged diffusely and linearly, were found in the cardiomyocytes, and occasionally diformazan granules were seen. The reaction in some cardiomyocytes was at the trace level.

In tests for LDH, SDH, and NADH-DH most cardiomyocytes revealed moderate and high activity, but also very high activity (SDH and LDH). The reaction product was mainly distributed linearly, and less frequently medium-sized granules of mono- and diformazan were arranged diffusely.

After intravenous irradiation with a helium—neon laser a tendency was observed for activity of all the dehydrogenases studied to increase. This was reflected in an increase in the number of myocardiocytes with higher activity. In the case of G6PDH (Fig. 1) the number of mono- and diformazan granules distributed linearly was increased in certain myocytes. Compared with the control, there was an increase in the number of myocytes with average activity. As regards the remaining dehydrogenases, there was an increase in the number of myocytes containing diformazan and characterized by very high (SDH and LDH) and high (NADH-DH) activity. On the whole, after intravenous helium—neon irradiation a tendency was noted for the activity of all enzymes studied to be increased on account of an increase in the number of cardiomyocytes in the population with higher activity than in the control.

After direct irradiation in vitro of parts of the myocardium with a helium—neon laser, a tendency also was observed for enzyme activity to be increased, although the tendency was weaker, especially for SDH and NADH-DH, activity of which in some cases was virtually identical with the control. This was expressed as an increase in the number of cardiomyocytes with a higher formazan content. In the case of G6PDH, the number of mono- and diformazan granules was increased, whereas in the case of LDH (Fig. 2) and other dehydrogenases, the diformazan content in individual cardiomyocytes was increased. In this case the distribution of formazan was similar in principle to that observed after intravenous irradiation.

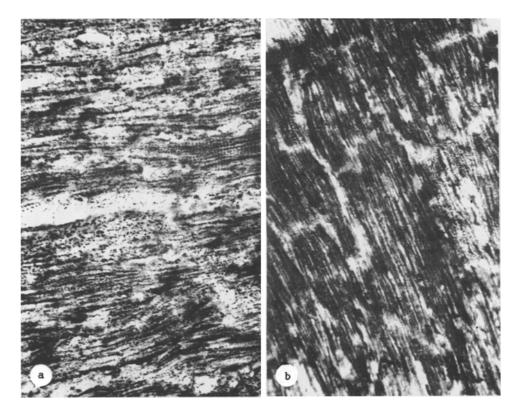


Fig. 2. Histochemical detection of LDH: a) control; b) after laser irradiation of myocardium in vitro. 400×.

Thus intravascular irradiation of blood with a helium—neon laser and direct irradiation of the myocardium in vitro lead to changes in activity of the enzymes studied, that are similar in direction although more marked after intravascular irradiation. This fact evidently reflects a difference in the mechanisms responsible for these changes.

According to data in the literature, during intravascular irradiation of blood with a helium—neon laser, activation of oxidative systems takes place [1] and the partial pressure of oxygen in the tissues rises [14]. These changes are accompanied by a parallel increase in release of biogenic amines [7], which influence virtually all types of metabolism, including electrolyte exchange. These changes may also lead to changes in the myocardial enzyme systems and, in particular, dehydrogenase systems.

On the other hand, direct irradiation of cells with a helium—neon laser increases cell membrane permeability and leads to changes in the sodium potassium balance [5] and, consequently, it changes the accessibility of substrates for intracellular enzyme systems.

The effects of helium—neon laser irradiation described above suggest that the changes we found in dehydrogenase activity are due to the effect of the helium—neon laser both directly on the cardiomyocytes and indirectly through the blood, including biogenic amines.

Intravascular irradiation of blood with a helium—neon laser and direct irradiation of the myocardium of albino rats thus lead to parallel activation of G6PDH, LDH, SDH, and NADH-DH. In the case of direct irradiation of the myocardium these changes are less marked, and for SDH and NADH-DH they are absent altogether. It can be tentatively suggested that the helium—neon laser has a certain activating effect on enzymes of myocardial energy metabolism.

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## EFFECT OF NERVE GROWTH FACTOR ON CHOLINERGIC NEURONS IN DISSOCIATED CULTURES OF THE SEPTUM PELLUCIDUM

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In recent years investigations of trophic factors, with the primary aim of studying their importance for morphogenesis, viability, and regeneration of the neurons of the developing brain, have expanded greatly. Nerve growth factor (NGF), discovered as long ago as in the 1950s by Levi-Montalcini and Hamburger [10], and characterized by them as a trophic factor of peripheral and noradrenergic sympathetic and sensory neurons, has been studied particularly intensively in this respect.

It has now been shown that a comparatively large quantity of NGF is present in certain structures of the CNS (neocortex, hippocampus) [3, 8], and that if injected into the cortex, it is transported into cholinergic neurons (ChN) of the basal nuclei of the forebrain [9]. Studies of the whole brain and cell cultures have shown that NGF affects the viability of ChN and the level of activity of choline-acetyltransferase and acetylcholinesterase (AChE) [5-7, 11].

In the investigation described below the effects of NGF were studied on body size and on the number of ChN in dissociated cell cultures of the rat embryonic septum pellucidum.

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